AMENDMENTS TO THE SPECIFICATION

IN THE SPECIFICATION:

Please insert after page 25, but before the claims, the attached paper Sequence Listing in the specification.

Attachments: Sequence Listing (paper copy)
Sequence Listing (computer readable copy, (CD-R)).

Please amend page 9 lines 24- page 10 line 2 as follows:

Figure 2 shows a bar diagram that depicts the specific antibody response of four groups of mice that had been immunized with recombinant Tat 1-72 (SEQ ID NO:1) alone (a), and in combination with Alum (b), HPC (c), and HPC and Alum (d), according to the protocol described in Example 1 (a).

Figure 3 shows a bar diagram that depicts the induction of Tat 1-72 (SEQ ID NO:1) specific T cell responses from mice that had been immunized with Tat 1-72 (SEQ ID NO:1). T cell proliferation was determined in the absence or the presence of Tat 1-72 (SEQ ID NO:1) at a concentration of 1 μg/ml or 10 μg/ml, according to the protocol described in Example 1 (b).

Figure 4 shows the result of an RNAse protection assay that was performed according to the protocol outlined in Example 1 (c). The protected RNA fragments correspond to the cytokines that are expressed by Tat immune T cells in response to 1 μg/ml Tat 1-72 (SEQ ID NO:1).

Please amend page 12 lines 1 – page 13 line13 as follows:

The present inventors have surprisingly discovered that recombinant Tat protein tightly binds bacterial RNA, which masks Tat epitopes. Figure 1 shows Tat binding to RNA in samples analyzed by agarose gel electrophoresis followed by staining with ethidium bromide. The absence of signal in lane 4 suggests that the Tat protein binds to RNA which has the molecular mass of transfer RNA (1200bp). The present inventors have further discovered that Tat protein copurifies with endotoxin, which may mask Tat epitopes, and/or suppress immune function. In a preferred embodiment a recombinant Tat protein is prepared that does not have RNA tightly bound to it, and that is substantially free of endotoxin contamination.

The present inventors have discovered that wild-type Tat 1-72 (SEQ ID NO: 1) is capable of inducing significant and specific humoral and T cell mediated immune responses, and that Tat 1-72 is not immunosuppressive *in vivo*. The term "wild-type" refers to an amino acid sequence encoded by a cDNA that is identical to that encoded by the endogenous gene. Tat refers to the protein product of the *tat* gene. In still another aspect of the present invention there is a recombinant Tat 1-72 (SEQ ID NO: 1) that faithfully reproduces both the primary and tertiary structure of naturally occurring, wild-type Tat 1-72 (SEQ ID NO: 1).

While the specific embodiments set forth below are directed to HIV-1, the purification and therapeutic methodologies set forth herein are equally applicable to HIV-2, owing to the recognized homology between the two viral strains. As used in the general discussion of the invention, "HIV" refers to HIV-1 or HIV-2, unless otherwise specified.

Preferably, some embodiments of the present invention provide methods for using a recombinant Tat protein for inducing humoral and cellular responses in an animal, preferably a mammalian animal, and more preferably a human. More preferably, the Tat protein is autonomously internalized by cells; that is, not integrated into the host genome. As mentioned above, the second exon of Tat influences the tertiary configuration of Tat and greatly potentiates Tat cellular uptake. Three forms of recombinant Tat proteins were evaluated for their immunogenic effects: Tat 1-86 (SEQ ID NO: 2), Tat 1-72 (SEQ ID NO: 1), and mutated Tat 1-86 (mTatl-86) (SEQ ID NO: 3). Tat 1-72 (SEQ ID NO: 1) protein was derived from the HIV-1 BRU exon 1; and Tat 1-86 (SEQ ID NO: 2) was derived from HIV-1 BRU exons 1 and 2. The HIV-1 BRU exons were obtained from Dr. Richard Gaynor through the AIDS repository at the

NIH. Mutated Tat 1-86 (mTat 1-86) (SEQ ID NO: 3), which is derived from exon 1, was modified to contain a single amino acid substitution at position 22, whereby Cys 22 was substituted by a Gly using site directed mutagenisis (Rossi, C. et al. 1997 Gene Ther. 4:1261; Caselli, E. et al. 1999 J. Immunol. 162:5631). The protein mTat 1-86 is a transdominant Tat mutant that lacks HIV-1 transactivation activity, and has been shown to elicit immune response against wild-type Tat protein in a mouse model.

The method for preparing recombinant proteins has been described previously by the inventors (Ma, M. and Nath, A. 1997 J. Virol. 71:2495; Nath, A. et al. 1996 J. Virol. 70:1475; Nath, A. et al. 1996 J. Neurovirol. 2:17; Holden, C.P. et al. 1999 Neuroscience 91:1369; Haughey, N.J. et al. 1998 J. Neurovirol. 4:353; Nath, A. et al. 2000 Ann. Neurol. 47; Haughey, N.J. et al. 1999 J. Neurochem. 73:1363). Briefly, wild-type Tat 1-72 (SEQ ID NO: 1) and Tat 1-86 (SEQ ID NO: 2), and mTat 1-86 (SEQ ID NO: 3) were subcloned into a bacterial vector PinPoint Xa-2(Promega) to express Tat as fusion proteins that are naturally biotinylated at the N-terminus. *E. Coli* bacteria were transformed with the resulting vector, and were grown in 200 ml of Luria Broth for 18 hours and in 2 L of Terrific Broth for 1 hour. The cells were harvested, and lysed, and the biotinylated Tat protein was purified by affinity chromatography using a soft release avidin resin. Tat was cleaved from the fusion protein by enzymatic cleavage using factor Xa, eluted and desalted using a PD 10 column. It is obvious to those skilled in the art that the genomic and amino acid sequence and length of Tat varies amongst different strains of HIV. Also, the protein could be produced in various strains of *E. coli*, other organisms or cells.